BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AND NITROGEN LEACHED OR EXTRACTED FROM PASTURE SOILS

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Abstract

Soils under grazed pastures from temperate regions tend to lose significant amounts of C and N as dissolved organic carbon (DOC) and nitrogen (DON) through leaching or runoff. Literature suggests 100 to 1600 kg C ha^{-1} yr^{-1} as DOC and 5 to 120 kg of N as DON ha^{-1} yr^{-1} can be lost through leaching from some New Zealand pasture soils. If these compounds are amenable to microbial decomposition then this could have important environmental consequences. We have examined microbial decomposition of DOC and DON from pasture soils. We also examined the effects of adding cow dung and urine on DOC and DON in soil solution and the subsequent decomposition of these fractions of dissolved nutrients following laboratory incubation studies where concentrations of DOC, DON, NH_4, NO_3 were measured at various time intervals up to 49 days. Loss of dissolved C through microbial respiration (CO_2) and N as N_2O were also measured periodically.

The DOC concentrations in cold-water (20°C for 30 minutes) extracts ranged between 29 and 148 ppm in solution and DON concentration ranged between 2 and 95 ppm. Spiking soils with dung increased both DOC and DON concentrations whereas urine had very little effect on DOC but a significant (P<0.001) effect on DON concentration. Significantly (P<0.001) higher concentrations of DOC and DON were extracted with hot-water (80°C for 16 hrs). Between 16 and 60% of the total DOC in the water extracts was respired as CO_2 by day 36. The hot-water extractable DOC was most rapidly respired (60% of the total) compared to other treatments (16-35%). In contrast to DOC, DON concentration in the extracts declined rapidly. Within the first 13 days of incubation, the concentration of DON was near zero without any change in the concentration of NO_3 or NH_4, indicating microbes utilised the organic pool of N preferentially. Decomposition of leached DOC and DON from lysimeters followed a similar pattern to that observed with soil extracts. Approximately 15 to 45% of the DOC in leachates was respired as CO_2 at day 49. The concentration of DON in the leachates declined to below 1 ppm within 7-14 days of the incubation, again consistent with the observations made with extractable DON. Our results clearly show that DOC and DON components of the DOM, whether extracted or leached, are highly decomposable and bioavailable. These findings warrant more in-depth studies to understand the role of DOM in regulating the ecosystem functions and nutrient balance in grazed pasture systems.

Introduction

The general perception is that leachable dissolved organic matter (DOM) which includes carbon and nitrogen (DOC and DON) from agricultural soils are predominantly recalcitrant; hence DOM movement from soil beyond the rooting depth and into ground water is considered to be of little concern environmentally. The importance of DOM in many soil processes is determined in large part by its availability for microbial uptake and decomposition, as this biodegradation can provide both energy to support microbial growth and limiting nutrients (McDowell et al. 2006). Microbial decomposition of DOM can regulate

the production of greenhouse gases such as CH_4 and N_2O both by reducing the O_2 content of soils and by providing the electron required for methonogenesis and denitrification. Soils from temperate regions under grazed pastures can lose significant amounts of C and N as DOC and DON through leaching or runoff. Literature suggests 100 to 1600 kg C ha^{-1} yr^{-1} as DOC and 5 to 120 kg of N as DON ha^{-1} yr^{-1} can be lost through leaching from some New Zealand pasture soils (Ghani et al. 2007; Ghani et al. 2010). If these compounds are amenable to microbial decomposition then this could have important environmental consequences. Lack of quantitative data on the biodegradability of DOC and DON from agricultural soils is being affected by lack of suitable methodologies. This study examined the decomposition of extractable and leachable DOC and DON from pasture soils using the soil microbial biomass assay by measuring disappearance of DOC and DON in solution as well as quantifying CO_2 production.

Materials and Methods

Extractable DOM

Soil samples (0-10 cm depth) were collected from allophanic and pumice soils under permanent pastures. Some pertinent soil biochemical characteristics of these soils are shown in Table 1. The DOM was extracted from field moist soils by cold-water (20°C for 30 minutes) and hot-water (80°C for 16 hrs) procedures as described by Ghani et al. (2003). The soil to water ratio during extraction was maintained at 1:5. The concentration of DOC in the extracts was measured by Shimadzu TOC analyser and the concentrations of NO_3, NH_4 and DON were measured following the method described by Ghani et al. (2007).

In order to get higher concentration of DOM in the extracts, these soils were also spiked with urine (5 ml/kg soil) and dung (2 g dry weight). The spiked soils were left for 7 days at 20°C to equilibrate then extracted with cold-water using the procedure described above. Concentrations of different components of DOM are shown in Table 2.

Table 1: Some chemical properties of the soils.

Soil properties	Allophane	Pumice
Total soil carbon % (w/w)	6.40	10.70
Total soil nitrogen % (w/w)	0.57	0.70
Soil pH	5.2	5.2
Water extractable carbon (µg OC/g soil)	57.8	95.1
Hot-water extractable carbon (µg OC/g soil)	2640	4620
Water soluble nitrate (µg NO_3/g soil)	37.6	31.0
Water soluble ammonium (µg NH_4/g soil)	1.0	0.3

Table 2. Concentrations of dissolved organic C and N and nitrate and ammonium in soil extracts. All concentrations are parts per million (mg/L).

Extracts	DOC	DON	NO_3	NH_4
Horotiu				
Cold-water (CW)	29	2	19	0.5
Hot-water (HW)	87	6	1	1.1
Urine + Soil	33	18	140	6.5
Dung + Soil	65	8	16	0.1
Taupo				
Cold-water (CW)	48	3	15	0.1
Hot-water (HW)	154	10	1	0.6
Urine + Soil	38	63	108	21.9
Dung + Soil	148	28	19	0.4

Leachable DOM

Leachable DOM was collected from an outdoor large lysimeters facility situated at the Ruakura Research Centre, Hamilton where replicated (4) intact cores (50 cm wide and 80 cm depth) from permanent pastures located in Northland, Rotorua, Hamilton and Tokanui were brought in and installed at the site in the spring of 2009. Leachates collected in May 2010 were used for the bio-decomposition study. Concentrations of DOC, DON, NO_3 and NH_4 in the leachates were measured (Table 2) prior to starting the decomposition study.

Table 3: Concentration of dissolved organic C, N, nitrate and ammonium in leachates collected from lysimeters. All values are reported as ppm (mg/L).

Soils	DOC	DON	NO_3	NH_4
Northland	66	7	12	0.1
Rotorua	9	6	25	0.1
Horotiu	7	11	38	0.2
Tokanui	8	1	3	0.0

Bio-decomposition set up

A volume of 200 ml of either the extracted or leached DOM was added into modified Schott bottles, which were air tight but had outlets fitted with septa for periodic gas and liquid sub-sampling. A trace element solution minus any nitrogen (Zahn-Wellens method, 1999) and 1 ml of fresh soil extract (1:10 soil and water) used as microbial inoculants were added to each bottle; each treatment was replicated 4 times. The bottles were placed on a bench shaker at 30 shakes per minute at 20°C. In the first experiment which ran for 36 days, the concentration of DOC, DON, NH_4, and NO_3 at 0, 14, 20 and 36 days were measured. Loss of carbon through microbial respiration (CO_2) and N as N_2O were also measured. A positive control using

sodium acetate (Na_2COOH) at a concentration of 50 ppm C and ammonium nitrate (NH_4NO_3) at a concentration of 5 ppm N was also included to compare decomposition of soil DOM. The experiment was repeated and in the second experiment, sampling intervals were 0, 3, 7, 14, 34 and 49 days.

DOC respired as CO_2

The CO_2 concentrations in the samples were measured using an Agilent gas chromatograph (Model GC G1530A) fitted with a GS-GASPRO 60 m x 0.32 mm column (J&W Scientific Inc.) and thermal conductivity detector. One ml of gas samples were collected from the headspace of the sealed incubation bottles through one of the sampling ports. Each sample was manually injected into the GC inlet septum. Helium was used as a carrier gas flowing at 2.2 ml min^{-1}. The mixture of sample and carrier gas was passed through a J&W Scientific Inc's. The CO_2 peak had a retention time of approximately 4 minutes. The carbon dioxide standards prepared in nitrogen gas were used and the concentration of CO_2 in samples was calculated using ChemStation Rev B.02.01-SRI [260] software.

Results and Discussion

Concentrations of DOC, NO_3, NH_4 and DON in extracts

The mounts of DOC and DON extracted by cold and hot-water methods were affected by soil type. Taupo pasture soil had a 65% greater concentration of DOC than Horotiu soil (48 vs. 29 ppm) in the cold-water extracts. A similar difference was found in the hot-water extracts (Table 2). While there was very little difference between the two soils in the concentration of nitrate and ammonium extracted either by hot or cold-water, the concentration of DON extracted by hot-water from the Taupo soil was significantly greater than from the Horotiu soil.

Addition of dung or urine resulted in greater concentration of cold-water extractable DON, NO_3 and NH_4 in both soils. However, there was very little effect on DOC concentration in both soils when urine was added (Table 2). The reason for this is that, after spiking the soil with dung or urine, these soils were incubated for 7 days at 20°C and at a moisture content 75% of the water-holding capacity prior to extraction. So urine-C during this initial incubation could have been converted into CO_2 by microbial respiration.

Concentrations of DOC, NO_3, NH_4 and DON in leachates

Leachates collected at the first drainage event following summer in 2010 were used in this study. There was a wide ranging concentration of DOC and DON as well as NO_3 (Table 3). There was nearly 10 fold difference in the DOC and DON concentrations in the leachates collected from different soils. Leachates collected from Northland and Horotiu soils had the highest and lowest concentrations of DOC, respectively (Table 3). The highest and the lowest concentrations of DON were measured in the Horotiu and Tokanui soils, respectively. Because DON concentration is calculated by subtracting concentrations of NO_3 and NH_4 from the total N in solution the instrumental detection limits associated with measurements of these three components of N can lead to over or under estimation of DON by 2 ppm. Therefore, when the concentration of DON is below 2 ppm it is not possible to study its bio-decomposition over time.

Decomposition of extractable DOC and DON

The rate of disappearance of DOC in the water extracts varied between soil solutions. By day 36 almost all the C (50 ppm) in the control treatment had disappeared from the incubated solution (Fig. 1a,b). Hot-water extractable DOC from both soils was the most highly bio-

decomposable followed by DOC extracted after spiking the soil with dung. The rates of disappearance of DOC in the other extracts such as urine spiked or cold-water extractable DOC were considerably slower. The loss of C from the DOC pool in soil extracts mirrored the recovery of C measured as CO_2 in the incubation bottle headspace (Fig. 1.c,d). Between 85 and 95% of the total C originally in the soil solutions was accounted for by adding the C remaining in the solutions after incubation to that which was respired as CO_2. The rates of C disappearance and respiration indicated between 20 and 60% decomposition of the DOC in the soil extracts. These ranges of decomposition of DOC are consistent with those observed by McDowell et al. (2006) who reported between 10 and 60% decomposition of DOC from soil extracts. Rates of respirations were faster in the first 21 days of incubation and dropped thereafter which is also consistent with the observations of Gregorich et al. (2003). As soil extracts are a mixture of easily biodegradable and refractory type of compounds, it is not surprising to see that the respiration rate and rate of carbon disappearance drops after 21 days.

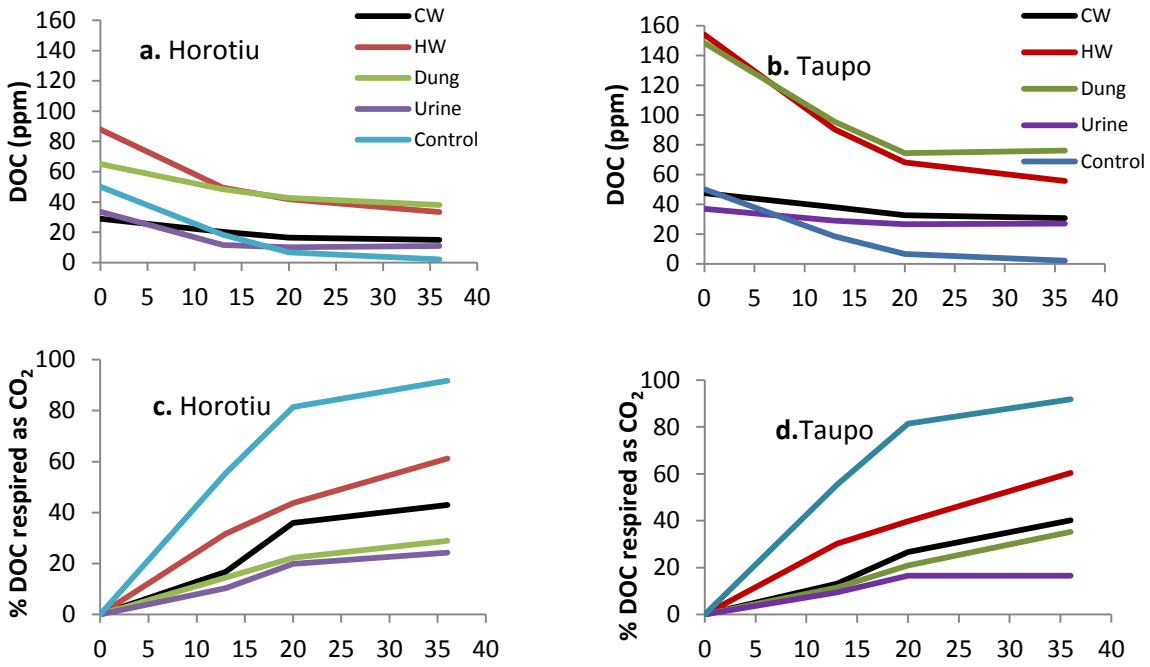

Figure 1: Changes in dissolved organic carbon extracted from Horotiu and Taupo soils and rates of dissolved carbon being respired during the incubation period.

The rate of disappearance of DON in solution was rapid in all treatments except the urine spiked extracted DOM from the Horotiu soil which was the least bioavailable (Fig. 2a). In all other treatments, DON concentration declined significantly by day 21 (Fig. 2). The rate of disappearance of DON was higher in the Taupo than the Horotiu soil extracts. Over 50% of the DON concentration from both the urine and the dung treated soil extracts disappeared within 13 days of incubation. Given that the starting concentrations of DON in these treatments were considerably high i.e. 63 and 28 ppm, respectively, then the fact that these declined to 15 and 10 ppm by day 13, showing how quickly this pool of N can be utilised by microbes. By day 36 most of the DON had disappeared from the soil solution except in the urine spiked extracts. Lack of bioavailable C in this treatment may have prevented any use of DON by microbes. It is interesting to note while DON concentration declined, there was very little change in the concentration of nitrate in any of the treatments (Fig. 2 c and d). There

were however, fluctuations in the concentration of ammonium during the incubation. The most noticeable change occurred in the hot-water extracts in both soils which tended to increase with time suggesting some conversion of DON into NH_4 (Fig. 2 e and f). These results clearly demonstrate that a large proportion of the extractable DON is highly bioavailable. Given that the nitrate concentrations in all treatments remained relatively static during the incubation period, it can be concluded that, from microbial biomass perspective, DON was the preferred source of N in this case, followed by NH_4. This opens a question as to whether DON has any regulatory role in immobilisation of nitrate-N in soil solution.

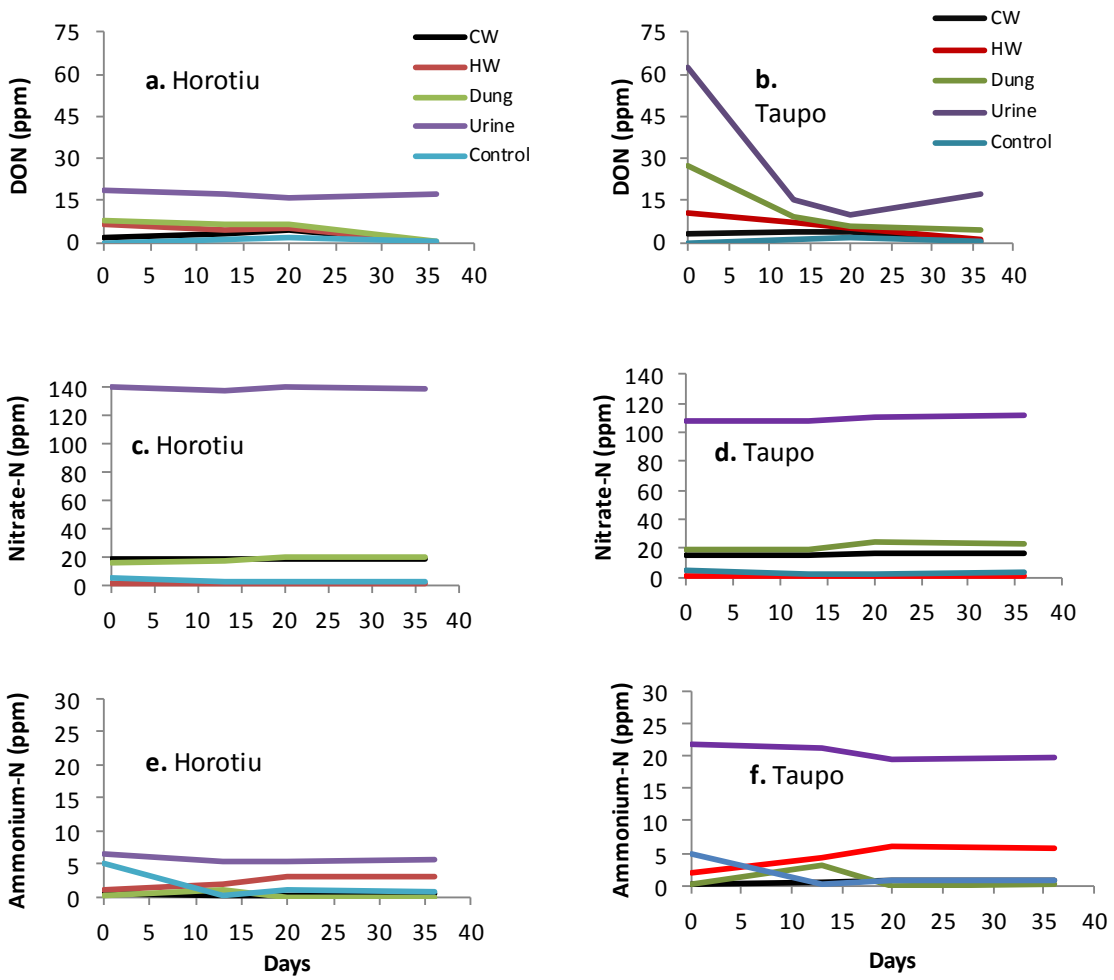

Figure 2: Changes in the extractable dissolved nitrogen, nitrate and ammonium in the incubated soil extracts.

With the same amounts of addition of dung or urine to both soils, there was more extractable DOC and DON in the Taupo soil than in the Horotiu. This could be due to a lower buffering capacity of the Taupo soil leading to more soluble material available in the soil solution or a difference in the microbial biomass, where added soluble C through dung or urine was quickly respired by microbes in the Horotiu soil than Taupo soil.

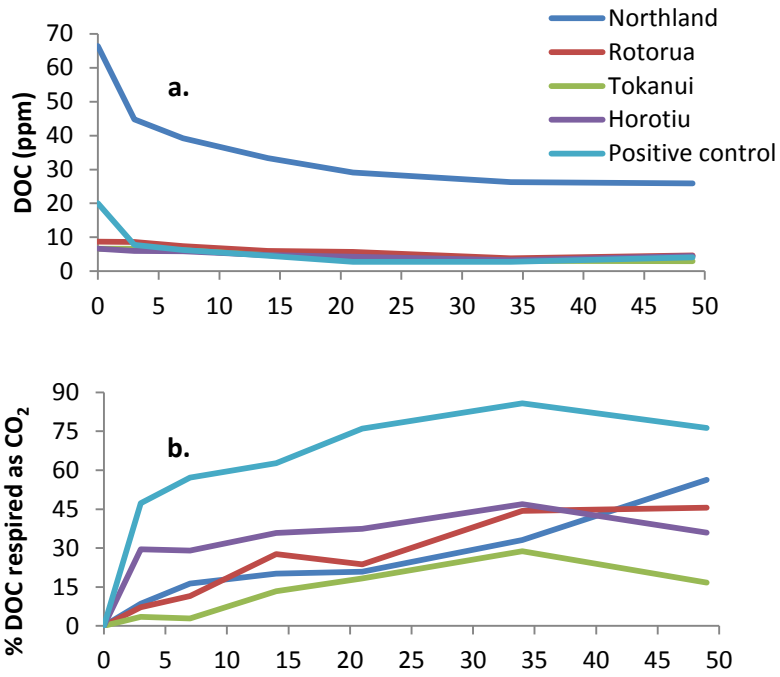

Figure 3: Changes in dissolved organic carbon concentration as well its respiration from leachates drained from different soils.

Decomposition of leached DOC and DON

Results shown in Fig. 3 a and b demonstrate that DOC in leachates was highly bioavailable. The concentration of DOC declined rapidly within the first 3-7 days. After 21 days, the decomposition of DOC slowed down and by the conclusion of the experiment (49 days) between 35 and 55% of the DOC in the leachates had been respired as CO_2. Again these findings are consistent with results reported for soil extracts and those reported by McDowell et al. (2006) and Gregoritch et al. (2003). The remaining proportion of the DOC in solution was either less bioavailable or its decomposition was limited by some other biochemical constraints such as availability of other nutrients. Over the same time period, approximately 85 and 90% of the C from control was respired. Like the soil extracts, most of the C was accounted for when the C remaining in solution and that which was respired was added together. Between 2 and 10% appeared to have been locked in the microbial biomass which was growing in the incubation bottles. Like the observation made with extractable DON, the leached DON was also highly bioavailable. Most of the DON concentrations were near zero ppm by day 13 (Fig. 4). Over the same time period, the concentration of nitrate-N remained static. Again, these observations are consistent with what was observed with soil extracts. These observations are consistent with studies conducted by Seitzinger et al. (2002) and (2005) using estuarine plankton communities as biological indicators which showed high bioavailability of DON originating from urban, animal and pastures and forestry operations. Our study now adds to the body of evidence emerging to suggest that DON is a highly bioavailable pool of N in soil-plant ecosystem. Its role in nitrogen cycling requires further investigation.

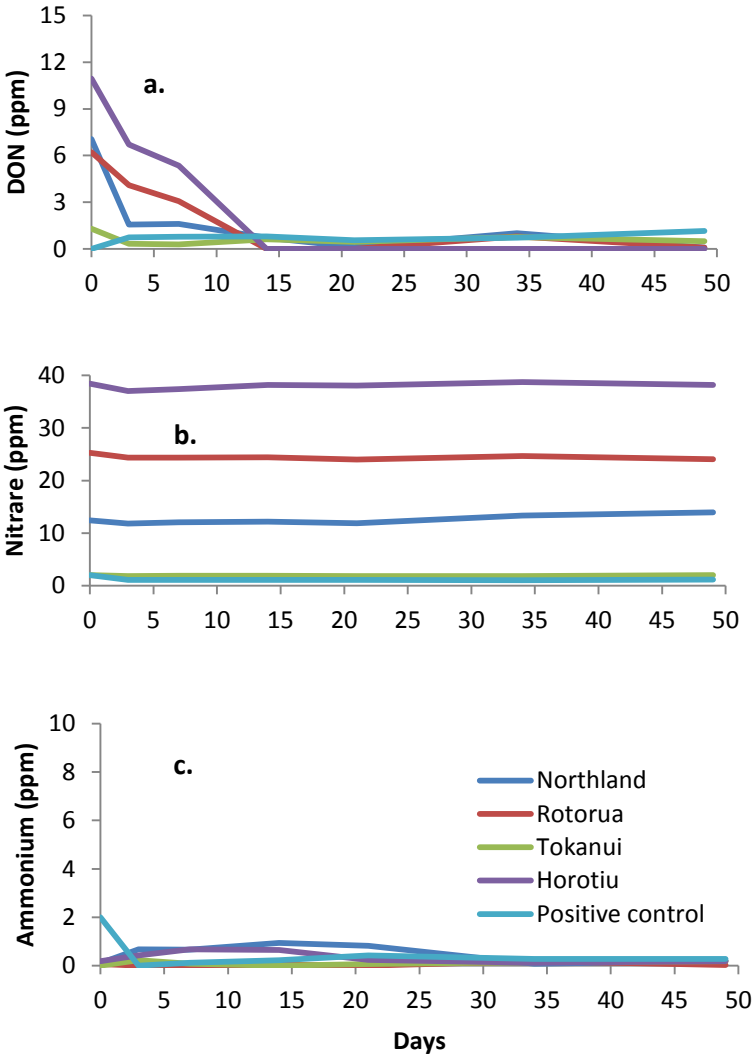

Figure 4: Changes in dissolved organic nitrogen, nitrate and ammonium concentration from leachates drained from different soils during the incubation period.

Conclusions

Our studies demonstrate that nutrients in DOM from pasture land use are highly bioavailable. Both extractable and leachable DOC and DON were found to be rapidly utilised by microbial biomass. Nearly 50% of the extractable or leachable DOC and the entire DON was highly bioavailable. Even in the presence of nitrate and ammonium-N, in all treatments microbes preferentially utilised DON component of the soluble N. These results are consistent with findings by others in estuarine environment. In a pasture land use system, role of DON in ecological context need to be investigated. Further research is needed to ensure loss of DON is accounted for in nutrient balance models.

Acknowledgements

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References

Ghani A, Dexter M, and Perrot KW, (2003) Hot-water extractable carbon in soils; a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry, 35:1231-1243.

Ghani A, Dexter M, Carran A, and Theobald TW, (2007) Dissolved organic nitrogen and carbon in pastoral soils; the New Zealand experience. European Journal of Soil Science, 58:832-843.

Ghani A, Muller K, Dodd M, Mackay A, (2010) Dissolved organic matter leaching in some contrasting New Zealand soils. European Journal of Soil Science 61:525-538.

Gregorich EG, Beare MH, Stoklas U, St-Georges P, (2003) Bioavailability of soluble organic matter in maize-cropped soils. Geoderma 113:237-252.

McDowell WH, Zsolnay A, Aitkenhead-peterson, JA, Gregorich EG, Jones DL, Jodemann D, Kalbitz K, Marschner B and Schwesig D, (2006) A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry, 38:1933-1942.

Seitzenger SP, Hartnett H, Lauck R, Mazurek M, Minegishi T, Spyres G, and Styles R, (2005) Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. Limnology and Oceanography, 50(1):1-12.

Seitzinger SP, Sanders RW, Styles R, (2002) Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnology and Oceanography, 47(2):353-366.

Zahn-Wallens Test (1999) Water quality – evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Static test. The European Standard EN ISO 9888:1999.